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APPLICATION

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METHODS OF ALLEVIATING NEUROPATHIC PAIN

FIELD OF THE INVENTION

This invention relates generally to the field of pain therapy and more specifically to the use of prosaposin receptor agonist for the treatment of neuropathic pain.

CLAIM OF PRIORITY

This application is a continuation-in-part of U.S. Serial No. 08/611,307, filed on March 5, 1996 and International application PCT/US97/04143, filed March 5, 1997.

BACKGROUND OF THE INVENTION

Neuropathic pain results from injury to a nerve. In contrast to the immediate pain (nociceptive pain) caused by tissue injury, neuropathic pain can develop days or months after a traumatic injury. Furthermore, while pain caused by tissue injury is usually limited in duration to the period of tissue repair, neuropathic pain frequently is long-lasting or chronic. Moreover, neuropathic pain can occur spontaneously or as a result of stimulation that normally is not painful.

The clinical causes of neuropathic pain are widespread and include both trauma and disease. For example, traumatic nerve compression or crush and traumatic injury to the brain or spinal cord are common causes of neuropathic pain. Furthermore, most traumatic nerve injuries also cause the formation of neuromas, in which pain occurs as a result of aberrant nerve regeneration. In addition, cancer-related neuropathic pain is caused when tumor growth painfully compresses adjacent nerves, brain or spinal cord. Neuropathic pain also is associated with diseases such as diabetes or alcoholism.

Unfortunately, neuropathic pain frequently is resistant to available drug therapies. In addition, current therapies have serious side-effects including, for example, cognitive changes, sedation,

nausea and, in the case of narcotic drugs, addiction. Many patients suffering from neuropathic pain are elderly or have other medical conditions that particularly limit their tolerance of the side-effects associated with available drug therapy. The inadequacy of current therapy in relieving neuropathic pain without producing intolerable side-effects frequently is manifest in the depression and suicidal tendency of chronic pain sufferers.

Methods of alleviating neuropathic pain would improve the quality of life for many people suffering from pain due to trauma or disease. However, there currently are no effective drugs that relieve neuropathic pain without undesirable side-effects such as sedation and addiction. Thus, there is a need for methods of alleviating neuropathic pain without producing undesirable side-effects. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method of alleviating neuropathic pain in a subject by administering a neuropathic pain alleviating amount of a prosaposin receptor agonist to the subject. For example, the invention provides a method of alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex in a subject by administering a neuropathic pain alleviating amount of a prosaposin receptor agonist having the amino acid sequence Cys-Glu-Phe-Leu-Val-Lys-Glu-Val-Thr-Lys-Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO:1) or Thr-D-Ala-Leu-Ile-Asp-Asn-Asn-Ala-Thr-Glu-Glu-Ile-Leu-Tyr (SEQ ID NO:2). In addition, the invention provides a method of inhibiting the onset of neuropathic pain in a subject by administering a neuropathic pain alleviating amount of a prosaposin receptor agonist to the subject. The present invention also provides prosaposin receptor agonists, prosaposin-derived peptides and the use of these peptides for stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination and inhibiting neural demyelination. In addition, there is provided a method of inhibiting sensory or motor neuropathy by contacting neuronal cells with a composition comprising a neuropathic pain alleviating amount of a prosaposin receptor agonist.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the threshold of tactile allodynia before (time 0) and at various times after bolus injection of prosaposin-derived 22-mer peptide (SEQ ID NO:1) in Chung model rats.

FIGURE 2 shows the threshold of tactile allodynia before (time 0) and at various times after bolus injection of prosaposin-derived 14-mer peptide (SEQ ID NO:2) in Chung model rats.

FIGURE 3 shows the sum flinches in response to 0.5% formalin after intraperitoneal administration of prosaposin-derived 14-mer peptide (SEQ ID NO:2) or saline in diabetic rats.

FIGURE 4 shows the effects of prosaptide TX 14(A) on the relief of neuropathy in a diabetic rat model. Prosaptide TX 14(A) prevented hypoalgesia in streptozotocin (STZ)-diabetic rats

FIGURE 5 shows the effects of diabetes and efficacy of a peptide fragment of prosaposin in treating diabetic nerve dysfunction. All three groups treated with prosaptide TX 14(A) showed significant decreases in weight loss in a dose dependent manner when compared to diabetic animals. Thermal response latency was restored to control non-diabetic levels in animals treated with 200 and 1000 μg prosaptide TX 14(A), while the 20 μg treatment group showed an intermediate response.

FIGURE 6 shows the effects of diabetes and efficacy of a peptide fragment of prosaposin in treating diabetic nerve dysfunction. Diabetic animals treated with prosaptide TX 14(A) at doses of 200 and 1000 μg/kg body weight showed enhanced motor nerve conduction velocities compared to the untreated diabetic group and diabetic animals receiving 20 μg/kg body weight. In addition, all prosaptide TX 14(A)-treated rats showed a slower loss of sensory nerve conduction velocity when compared to diabetic unheated animals.

FIGURE 7 shows that prosaptide TX 14(A) halts progressive slowing of sensory conduction and reverses hyperalgesia in diabetic rats. Prosaptide TX 14(A), given parenterally, reverses hyperalgesia in the diabetic rat. The anti-hyperalgesic properties of prosaptide are specific to diabetes rats rather than being a general effect on the fomalin test *per se*.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of alleviating neuropathic pain in a subject by administering a neuropathic pain alleviating amount of a prosaposin receptor agonist to the subject. As disclosed herein, the method of the invention can alleviate neuropathic pain in a subject within 30 minutes of administration. Such a method is useful for alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex.

As used herein, the term "prosaposin receptor agonist" refers to a molecule which binds to any site on a cell to which prosaposin or a prosaposin-derived molecule can bind, and which thereby acts to alter the cell's function in the manner that prosaposin or a prosaposin-derived molecule acts. An agonist is any molecule that improves the activity of a different molecule; *e.g.*, a hormone, which acts as an agonist when it binds to its receptor, thus triggering a biochemical response. A molecule that both binds to receptors and has an intrinsic effect is an agonist. A receptor agonist is a substance that mimics a specific hormone, is able to attach to that hormone's receptor, and thereby produces the same action that the hormone usually produces. Drugs are often designed as receptor agonists to treat diseases and disorders caused when the hormone is missing or depleted in a subject.

As used herein, the term "prosaposin receptor" refers to a site on a cell to which prosaposin or a prosaposin-derived molecule can bind, thereby acting to alter the cell's function. Prosaposin receptors may be cell surface proteins, other cell proteins, or glycosphingolipids. One putative prosaposin receptor protein is a 54-60 kilodalton (kDa) protein isolated from whole rat brain, rat cerebellum and mouse neuroblastoma cells using the plasma membrane P-100 fraction. The 54-60 kDa protein binds irreversibly to saposin C, a prosaposin derivative. The isolation of the putative prosaposin receptor is described in EXAMPLES XI and XII.

Prosaposin receptors may also be membrane lipids called glycosphingolipids. Glycosphingolipids are sphingolipids that have a carbohydrate head group and two hydrocarbon chains; one a fatty acid and the other a sphingosine derivative. Glycosphingolipids are important components of the myelin sheath, a structure which protects and insulates nerve fibers. Prosaposin binds glycosphingolipids such as gangliosides, cerebrosides and sulfatides with high affinity and facilitates their transfer from

micelles to membranes (Sueda *et al. J. Biol. Chem.* (1993); Hiraiwa *et al., Proc. Natl. Acad. Sci. USA., 89:* 11254-11258 (1992)). Gangliosides contain one or more sialic acid residues and are most abundant in the plasma membrane of neurons where they constitute approximately 6% of the total lipid mass. Although the function of gangliosides is largely unknown, they have been implicated in the stimulation of neuronal differentiation, neuritogenesis and nervous system repair.

In one embodiment, prosaposin receptor agonists may be prosaposin-derived peptides. As used herein, the term "active fragment of prosaposin" is synonymous with "prosaposin-derived peptide". A peptide useful in the invention is derived from prosaposin, which is a 517 amino acid protein originally identified as the precursor of four sphingolipid activator proteins (Kishimoto *et al., J. Lipid Res., 33:*1255-1267 (1992)). Four adjacent tandem domains in prosaposin are proteolytically processed in lysosomes to generate saposins A, B, C, and D, which activate hydrolysis of glycosphingolipids by lysosomal hydrolases (O'Brien and Kishimoto, *FASEB J., 5:*301-308 (1991)).

The unprocessed form of prosaposin is found in high concentrations in human and rat brain, where it is localized within neuronal surface membranes. During embryonic development, prosaposin mRNA is abundant in brain and dorsal root ganglia. Furthermore, prosaposin binds with high affinity to gangliosides, which stimulate neurite outgrowth, and promotes transfer of gangliosides from micelles to membranes.

The neurotrophic activity of prosaposin is consistent with its localization in neuronal cell populations (O'Brien *et al., Proc. Natl. Acad. Sci., USA 91:*9593-9596 (1994); Sano *et al., Biochem. Biophys. Res. Commun., 204:*994-1000 (1994)). Prosaposin stimulates motor neurite outgrowth *in vitro* and *in vivo* and increases choline acetyltransferase activity, which is a marker of neuronal differentiation. In addition, prosaposin prevents cell death in neuroblastoma cells (O'Brien *et al., supra*, 1994; O'Brien *et al., FASEB J. 9:* 681-685 (1995)).

The neurotrophic activity of prosaposin is localized to saposin C, a domain of 80 amino acids. A 22-mer peptide corresponding to amino acids 8 to 29 of the saposin C domain (SEQ ID NO:1)

stimulates neurite outgrowth and choline acetyltransferase activity and prevents cell death in neuroblastoma cells (O'Brien et al., *supra*, 1995).

Prosaposin or the prosaposin-derived 22-mer peptide (SEQ ID NO:1), for example, can modulate motor neuron function by promoting neurite outgrowth. Prior to the present invention, however, it was not known whether prosaposin or a peptide fragment of prosaposin could affect sensory neuron function. Moreover, the neurotrophic activity of prosaposin or a prosaposin-derived peptide in stimulating motor neurite outgrowth is evident only after a period of 24 to 48 hours (*see, for example,* O'Brien *et al., supra* (1994)). Neurotrophic activity of prosaposin or a prosaposin-derived peptide has not been demonstrated to occur in a shorter period of time.

In contrast, the present invention provides a method of alleviating neuropathic pain, which involves both sensory and motor neuron components. Furthermore, the method of the invention is effective in inhibiting or alleviating neuropathic pain in a matter of minutes rather than the hours or days previously demonstrated to be required for the neurotrophic activity of prosaposin or a prosaposin-derived peptide.

The effectiveness of the method of the invention in alleviating neuropathic pain was demonstrated using the well-recognized Chung rat model of peripheral neuropathy. In the Chung rat model, spinal nerve partial ligation of left spinal nerves L-5 and L-6 produces a long-lasting hypersensitivity to light pressure on the affected left foot. The hypersensitivity is similar to the pain experienced by humans with the neuropathic condition of causalgia as described in Kim and Chung, *Pain 50:*355-363 (1992).

Prior to administration of an active fragment of prosaposin, Chung model rats had a threshold of 3.0 to 4.0 g before the affected foot was withdrawn in response to pressure (Von Frey hairs) (*see* FIGURE 1). After administration of an active fragment of prosaposin (prosaposin-derived 22-mer; SEQ ID NO:1), neuropathic pain was alleviated, as evidenced by a greater tolerance to pressure before the affected foot was withdrawn. The effect of the active fragment of prosaposin occurred within 15 minutes and was sustained for 3 hours following administration as shown in FIGURE 1.

This rapid relief of neuropathic pain is in stark contrast to the delayed neurotrophic effects previously reported for prosaposin and peptides derived from prosaposin.

A prosaposin receptor agonist such as the prosaposin-derived peptide SEQ ID NO:2 also alleviated pain in a rat model of painful diabetic neuropathy. As described in EXAMPLE III, peptide SEQ ID NO:2 reduced allodynia in rats with short-term insulin-deficient diabetes induced by the selective β cell toxin, streptozotocin (STZ). Thus, a prosaposin receptor agonist of the invention can be used to alleviate a variety of types of neuropathic pain including mechanical pain, as exemplified by the Chung rat model, and metabolic pain, as exemplified by the use of these peptides in reducing pain in diabetic rats.

As used herein, the term "neuropathic pain" means pain resulting from injury to a nerve. Neuropathic pain is distinguished from nociceptive pain, which is the pain caused by acute tissue injury involving small cutaneous nerves or small nerves in muscle or connective tissue. Pain involving a nociceptive mechanism usually is limited in duration to the period of tissue repair and generally is alleviated by available analgesic agents or opioids as described in Myers, *Regional Anesthesia 20:*173-184 (1995).

Neuropathic pain typically is long-lasting or chronic and often develops days or months following an initial acute tissue injury. Neuropathic pain can involve persistent, spontaneous pain as well as allodynia, which is a painful response to a stimulus that normally is not painful. Neuropathic pain also can be characterized by hyperalgesia, in which there is an accentuated response to a painful stimulus that usually is trivial, such as a pin prick. Unlike nociceptive pain, neuropathic pain generally is resistant to opioid therapy (Myers, *supra* (1995)).

The method of the invention is useful in alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex. As used herein, the term "disorder" means any trauma, injury, disease or condition resulting in neuropathic pain.

The method of the invention is useful in alleviating neuropathic pain regardless of the etiology of the pain. For example, a method of the invention can be used to alleviate neuropathic pain resulting from a peripheral nerve disorder such as neuroma; nerve compression; nerve crush, nerve stretch or incomplete nerve transsection; mononeuropathy or polyneuropathy. A method of the invention also can be used to alleviate neuropathic pain resulting from a disorder such as dorsal root ganglion compression; inflammation of the spinal cord; contusion, tumor or hemisection of the spinal cord; tumors of the brainstem, thalamus or cortex; or trauma to the brainstem, thalamus or cortex (*see, for example,* TABLE 1).

The method of the invention can be useful, for example, to alleviate neuropathic pain resulting from a neuroma, which can develop readily after traumatic injury to nerve, especially when a whole nerve is severely crushed or transsected. In a neuroma, the neurite outgrowth that normally regenerates a peripheral nerve is aberrant or misguided due, for example, to a physical obstruction such as scar tissue. Thus, a regenerating nerve fiber is entangled in an environment in which mechanical and physical factors precipitate abnormal electrophysiologic activity and pain (Myers, *supra* (1995)). An amputation neuroma, for example, can cause phantom pain or can cause pain triggered by the use of a limb prosthesis. As disclosed herein, such neuropathic pain can be alleviated by administration of a prosaposin receptor agonist according to a method of the invention.

Nerve compression also results in neuropathic pain that can be treated using the method of the

TABLE 1
Nerve
Neuroma (amputation, nerve transsection)
Nerve compression (entrapment neuropathies, tumors)
Nerve crush, stretch or incomplete transsection (trauma)
Mononeuropathy
Diabetes mellitus
Irradiation
Ischemia
Vasculitis
Polyneuropathy
Post-polio syndrome
Diabetes mellitus
Alcohol
Amyloid
Toxic
HIV
Hypothyroidism
Uremia
Vitamin deficiencies
Chemotherapy (vincristine, cisplatinum, paclitaxel)
ddC (zalcitabine)
Fabry's disease
Dorsal root ganglion
Compression (disk, tumor, scar tissue)
Root avulsion
Inflammation (postherpetic neuralgia)
Spinal cord
Contusion
Tumor
Hemisection
Brainstem, thalamus, cortex
Infarction, tumors, trauma

invention. Nerve compression can be abrupt, as in the case of traumatic nerve crush, or can be prolonged and moderate, secondary to tumor growth or scar formation in the proximity of a major

nerve bundle. Compression neuropathy can occur as a result of changes in blood flow to a nerve, causing severe ischemia and consequent nerve injury (Myers, *supra* (1995)).

Administration of a prosaposin receptor agonist according to the method of the invention also can alleviate neuropathic pain resulting from a mononeuropathy or polyneuropathy. As used herein, a neuropathy is a functional disturbance or pathological change in the peripheral nervous system and is characterized clinically by sensory or motor neuron abnormalities. The term mononeuropathy indicates that a single peripheral nerve is affected, while the term polyneuropathy indicates that several peripheral nerves are affected.

The etiology of a neuropathy can be known or unknown (*see, for example,* Myers, *supra* (1995); Galer, *Neurology 45(suppl 9)*:S17-S25 (1995); Stevens and Lowe, *Pathology,* Times Mirror International Publishers Limited, London (1995)). Known etiologies include complications of a disease or toxic state; for example, diabetes is the most common metabolic disorder causing neuropathy. The method of the invention alleviates the neuropathic pain of a mononeuropathy resulting, for example, from diabetes, irradiation, ischemia or vasculitis. The method of the invention also alleviates the neuropathic pain of a polyneuropathy resulting, for example, from post-polio syndrome, diabetes, alcohol, amyloid, toxins, HIV, hypothyroidism, uremia, vitamin deficiencies, chemotherapy, ddC or Fabry's disease (*see* TABLE 1). The method of the invention particularly is useful in alleviating post-polio myalgia. The method of the invention also can alleviate neuropathic pain of unknown etiology.

As disclosed herein, a prosaposin receptor agonist, for example, an active fragment of prosaposin, also can be useful in alleviating neuropathic pain or in stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in inhibiting sensory neuropathy. The term "active fragment of prosaposin," as used herein, means a peptide that has an amino acid sequence corresponding to an amino acid sequence of prosaposin and that has activity in alleviating neuropathic pain or in stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in inhibiting sensory or motor neuropathy.

As used herein, the term "inhibiting neuropathic pain" or "alleviating neuropathic pain" refers to any diminution in the severity of neuropathic pain. In a human subject, a prosaposin receptor agonist reduces the severity of neuropathic pain such that the subject's suffering is diminished and quality of life is improved. A prosaposin receptor agonist, for example, an active fragment of prosaposin, also can alleviate neuropathic pain in any one of a number of well-established animal models of neuropathic pain as described further below (*also see* Bennett, *Muscle & Nerve 16:*1040-1048 (1993)). As used herein, the term "active fragment of prosaposin" is synonymous with "prosaposin-derived peptide".

In one embodiment, the prosaposin receptor agonist useful in the invention is a prosaposin receptor agonist of 14 to 50 amino acids ~~which has the amino acid sequence LIRX$_1$NNX$_2$TX$_3$X$_4$X$_3$X$_1$X$_1$, where X$_1$ is any amino acid, X$_2$ is any amino acid, but not L or R; X$_3$ is a charged amino acid; and X$_4$, when present, is a charged amino acid.~~

The prosaposin receptor agonist preferably contains the amino acid sequence Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO:3), which corresponds to amino acids 18 to 29 of saposin C. More preferably, an active fragment of prosaposin has the amino acid sequence Cys-Glu-Phe-Leu-Val-Lys-Glu-Val-Thr-Lys-Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO:1), which corresponds to amino acids 8 to 29 of saposin C, or the amino acid sequence Thr-D-Ala-Leu-Ile-Asp-Asn-Asn-Ala-Thr-Glu-Glu-Ile-Leu-Tyr (SEQ ID NO:2), which corresponds to amino acids 16 to 29 of saposin C but which has been modified by a D-alanine for lysine substitution at position 2; an alanine for lysine substitution at position 8; a deletion of lysine at position 11 and the addition of a C-terminal tyrosine residue (*see* TABLE 2). Such modifications can be useful for increasing peptide stability or uptake across the blood-brain barrier as described below. As used herein, D-alanine can be represented by D-Ala or X.

An active fragment of prosaposin can have about 12 amino acids to about 80 amino acids, which is the full-length of saposin C. Preferably, an active fragment of prosaposin has about 12 amino acids to about 40 amino acids and, more preferably, about 14 amino acids to about 22 amino acids.

TABLE 2		
PEPTIDE	**SEQUENCE**	**SEQ ID NO:**
Prosaposin-derived 22-mer	CEFLVKEVTKLIDNNKTEKEIL	1
Prosaposin-derived 14-mer	TXLIDNNATE-EILY	2
Prosaposin-derived 12-mer	LIDNNKTEKEIL	3
where X = D-alanine		

For use in alleviating neuropathic pain in a human subject, an active fragment of human prosaposin, such as SEQ ID NO:1 or SEQ ID NO:2, is preferred. However, an active fragment derived from another mammalian prosaposin also is useful in alleviating neuropathic pain according to the method of the invention. Thus, for example, an active fragment of mouse prosaposin, rat prosaposin, guinea pig prosaposin or bovine prosaposin such as SEQ ID NOS: 4 through 7 also can be useful in alleviating neuropathic pain in a subject.

The amino acid sequence of an active fragment of human prosaposin (SEQ ID NO:1), which corresponds to amino acids 8 to 29 of saposin C, is well conserved among other species, as shown in TABLE 3. In particular, adjacent asparagine (N) residues are conserved among human, mouse, rat, guinea pig and bovine prosaposins. In addition, a leucine (L) residue is conserved 3 to 4 residues toward the N-terminus of the two asparagine residues and one or more charged residues (aspartic acid (D), lysine (K), glutamic acid (E) or arginine (R)) are conserved 2 to 8 residues toward the C-terminus of the two asparagine residues. Each of these well-conserved residues is underlined in TABLE 3.

TABLE 3		
SPECIES	**SEQUENCE**	**SEQ ID NO.**
Human	CEFLVKEVTKLIDNNKTEKEIL	1
Mouse	CQFVMNKFSELIVNNATE-ELLY	4
Rat	CQLVNRKLSELIINNATE-ELL	5
Guinea Pig	CEYVVKKVMLLIDNNRTEEKII	6
Bovine	CEFVVKEVAKLIDNNRTEEEIL	7

The well-conserved adjacent asparagine residues, leucine residue and charged residues described above can be important for the activity of an active fragment of prosaposin in alleviating neuropathic pain or in stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in inhibiting or motor neuropathy. For example, the prosaposin-derived 22-mer (SEQ ID NO:1) or the prosaposin-derived 14-mer (SEQ ID NO:2) is a prosaposin receptor agonist, an active fragment of prosaposin, which reduces the painful allodynia seen in the Chung rat model of peripheral neuropathy, as disclosed in EXAMPLE I (*see* FIGURES 1 and 2). In contrast, a mutant 22-mer (SEQ ID NO:8), which differs from SEQ ID NO:1 in having an aspartic acid residue (D) in place of the first conserved asparagine (*see* TABLE 4), lacks activity in alleviating neuropathic pain as assayed using Chung rats (*see* EXAMPLE I).

TABLE 4		
PEPTIDE	**SEQUENCE**	**SEQ ID NO:**
Prosaposin-derived 22-mer	CEFLVKEVTKLIDNNKTEKEIL	1
Mutant 22-mer	CEFLVKEVTKLID**D**NKTEKEIL	8
Prosaposin-derived 14-mer	TXLIDNNATE-EILY	2
Mutant 14-mer M-1	TKLIDN**D**KTEKEIL	9
Mutant 14-mer M-2	TK**S**IDNNKTEKEIL	10
where X =D-alanine		

The activity of a peptide in alleviating neuropathic pain also can correlate with neurotrophic activity. For example, the prosaposin-derived 22-mer (SEQ ID NO:1) and the prosaposin-derived 14-mer (SEQ ID NO:2) alleviate neuropathic pain and have neurotrophic activity. In addition, the mutant 22-mer (SEQ ID NO:8) is inactive in alleviating neuropathic pain as described above and lacks neurotrophic activity, further indicating that activity in alleviating neuropathic pain can correlate with neurotrophic activity. The mutant 14-mer peptide M-1 (SEQ ID NO:9), which has a substitution of the second conserved asparagine residue, lacks neurotrophic activity, indicating that peptide SEQ ID NO:9 also is inactive in alleviating neuropathic pain. The mutant 14-mer peptide M-2 (SEQ ID NO:10), which has a substitution of the conserved leucine residue, lacks neurotrophic activity, indicating that peptide SEQ ID NO:10 is inactive in alleviating neuropathic pain. In contrast, the prosaposin-derived 12-mer peptide (SEQ ID NO:3), which has the conserved adjacent asparagines, leucine and charged residues described above, is active as a neurotrophic factor. Thus, the prosaposin-derived 12-mer peptide (SEQ ID NO:3) also can alleviate neuropathic pain according to the method of the invention.

Prosaposin receptor agonists, including prosaposin-derived peptides and neurotrophic analogs thereof, possess significant therapeutic applications in promoting functional recovery after toxic, traumatic, ischemic, degenerative or inherited lesions to the peripheral or central nervous system. In addition, these peptides can promote myelination or inhibit demyelination, thereby counteracting the effects of demyelinating diseases. Furthermore, such peptides stimulate the outgrowth of

neurons and inhibit programmed cell death in neuronal tissues. The active neurotrophic and myelinotrophic peptides of the invention have between about 12 or 14 and about 50 amino acids and preferably include the non-naturally occurring prosaposin sequence shown in SEQ ID NO:2. For example, the active neurotrophic and myelinotrophic peptides of the invention have between 14 and about 50 amino acids and include the non-naturally occurring prosaposin sequence shown in SEQ ID NO:2.

In another embodiment of the present invention, there is provided a method of stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination in differentiated or undifferentiated neuronal cells by administering to the neuronal cells an effective amount of a neurite outgrowth or myelin-facilitating peptide having between about 12 and about 50 amino acids and preferably including the peptide shown in SEQ ID NO:2. In the methods of the invention for stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination, an effective amount of a peptide having, for example, between 14 and about 50 amino acids and including the peptide shown in SEQ ID NO:2 can be used.

As used herein, the term "stimulating neurite outgrowth" refers to inducing or increasing the outgrowth of neural processes from neural cells. Neurite outgrowth may occur in differentiated or undifferentiated neural cells. For example, in differentiated cells, neurite outgrowth may be from from dorsal root ganglion explants, sympathetic ganglia explants, or nodose ganglion explants. Neurite outgrowth responses may also occur in neoroblastoma cells, such as NS20Y neuroblastoma cells or PC12 pheocromocytoma cells. As used herein, the term "inhibiting neural cell death" refers to the inhibition of the death of neural cells. Necrosis and apoptosis are two basic processes by which cells may die. In necrosis, cell death usually is a result of cell injury. The cells generally swell and lyse. The cell contents ultimately spill into the extracellular space. By contrast, apoptosis is a mode of cell death in which single cells are deleted in the midst of living tissues. Apoptosis accounts for most of the programmed cell death in tissue remodeling and for the cell loss that accompanies atrophy of adult tissues following withdrawal of endocrine and other growth stimuli. As used herein, the term "promoting myelination" refers to promoting the formation of a myelin sheath, a sheath of white, fatty protein (myelin) that covers and acts as an electrical insulator for

nerve fibers. Oligodendrocytes form myelin in the central nervous system. Schwann cell form myelin in the peripheral nervous system. As used herein, the term "inhibiting demyelination" refers to the inhibition of the destruction of myelin sheaths that surrounds nerve fibers, which results in the loss of function of those nerves. In several diseases, the body attacks its own nervous system, destroying the myelin sheath that protects the nerve cells. This demyelination prevents the nerves from carrying signals properly, and afflicted persons can experience problems with muscular coordination, vision and other sensory problems, and paralysis. Several diseases that result in demyelination of nerve fibers include multiple sclerosis, acute disseminated leukoencephalitis, progressive multifocal leukoencephalitis, metachromatic leukodystrophy and adrenal leukodystrophy.

The ability of any such peptide to stimulate neurite outgrowth, inhibit neural cell death, promote myelination or inhibit demyelination readily can be determined by one skilled in the art using the procedures described in EXAMPLES IV to VII. Methods for assaying the abilities of these peptides to promote myelination and to inhibit demyelination are set forth in EXAMPLES VI and VII below.

The present invention also provides a method of inhibiting sensory neuropathy by contacting neuronal cells with a composition comprising an effective inhibiting amount of a prosaposin receptor agonist, for example, an active fragment of prosaposin. The invention provides, for example, a method of inhibiting sensory neuropathy by contacting neuronal cells with a composition comprising an effective inhibiting amount of a peptide having the sequence shown as SEQ ID NO:1 or SEQ ID NO:2.

As described herein in EXAMPLE X, a prosaposin-derived peptide can be useful in inhibiting sensory neuropathy. In a mouse model in which sensory neuropathy is induced by taxol administration, a loss of thermal sensation is normally seen. However, in taxol-treated mice given 100 μg/kg of peptide SEQ ID NO:1, the loss of thermal sensation was inhibited. These results indicate that prosaposin-derived peptides can be a neurotrophic factor for both sensory and motor neurons.

A peptide useful in the methods of the invention also can be, for example, SEQ ID NOS:11 through 19 (*see* TABLE 5). For example, sequence alignment of the prosaposin-derived 22-mer peptide SEQ ID NO:1 with cytokines and growth factors indicates sequence similarity to a number of human (h) cytokines including hCNTF, hIL-6, hIL-2, hIL-3, hIL1-γ, erythropoietin (hEPO), human leukocyte inhibitory factor (hLIF), the hIL-1 β chain and oncostatin-M (hONC-M). SEQ ID NOS: 11 through 19, like the active fragment of prosaposin SEQ ID NO:1, contain two asparagine residues that are adjacent or separated by one amino acid. In addition, the cytokine-derived peptide sequences can contain a leucine (L) or isoleucine (I) residue three to four residues toward the N-terminus of the two asparagine residues and one or more charged residues (aspartic acid (D), lysine (K), glutamic acid (E), or arginine (R)) two to eight residues toward the C-terminus of the two asparagine residues, as is seen in the active fragment of prosaposin (22-mer; SEQ ID NO:1). Each of these residues is underlined in TABLE 5.

Models of cytokine-receptor binding (Sprang and Bazan, *Curr. Opin. Struct. Biol.*, *3*:816 (1993)) have highlighted the evolutionary conservation of a four-helical bundle structure common to many cytokines. Each of the cytokine or growth-factor sequences related to the prosaposin-derived sequence SEQ ID NO:1 is located between helices A and B (AB loop) or within helix C of the cytokine.

TABLE 5			
CYTOKINE	SEQUENCE	LOCATION	SEQ ID NO:
Prosaposin	CEFLVKEVTKLIDNNKTEKEIL	----	1
hCNTF	YVKHQGLNKNINLDSVDGVP	AB loop	11
hIL-6	EALAENNLNLPKMAG	AB loop	12
hIL-2	LQMILNGINNYKNPKLT	AB loop	13
hIL-3	ILMENNLRRPNL	AB loop	14
hIL1-γ	FYLRNNQLVAGTL	AB loop	15
hEPO	AEHCSLNENITVPDTKV	AB loop	16
hLIF	YTAQGEPFPNNVEKLCAP	AB loop	17
hIL-1β	FNKIEINNKLEFESA	Helix C	18
hONC-M	RPNIGLRNNIYCMAQLL	Helix C	19

The structurally related cytokine and growth factor-derived peptides SEQ ID NOS: 11 through 19 also can be useful in methods of alleviating neuropathic pain. Peptides SEQ ID NOS: 11 through 19 can be assayed for activity in alleviating neuropathic pain using, for example, the Chung rat model described in EXAMPLE I; a model of diabetic neuropathy as described in EXAMPLE III assays described by Wall *et al., Pain 7:*103-113 (1979); Bennett and Xie, *Pain 33:*87-107 (1988); Lekan *et al., Soc. Neurosci. Abstr. 18:*287 (1992) or Palacek *et al., Soc. Neurosci. Abstr. 18:*287 (1992); or other assays for neuropathic pain.

The cytokine and growth factor-derived peptides SEQ ID NOS: 11 through 19 also can be useful in methods of stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in methods of inhibiting sensory or motor neuropathy. A peptide having between about 14 and about 50 amino acids and including the active neurotrophic region contained within one of sequences SEQ ID NOS: 11 through 19 can be assayed for the ability to stimulate neurite outgrowth as described in EXAMPLE IV; or assayed for the ability to inhibit neural cell death as described in EXAMPLE V; or for the ability to promote myelination as described in

EXAMPLE VI; or for the ability to inhibit demyelination as described in EXAMPLE VII; or for the ability to inhibit sensory neuropathy as described in EXAMPLE X.

A prosaposin receptor agonist useful in alleviating neuropathic pain can be identified by screening a large collection, or library, of random peptides or peptides of interest using, for example, one of a number of animal models of neuropathic pain. Such prosaposin receptor agonists of interest can be, for example, the cytokine and growth factor-derived peptides SEQ ID NOS:11 through 19, which have amino acid sequences related to an active fragment of prosaposin (SEQ ID NO:1). Peptides of interest also can be, for example, a population of peptides related in amino acid sequence to SEQ ID NO:1 by having the conserved asparagine residues, leucine/isoleucine residue and one or more charged residues at the positions corresponding to the positions in which these residues are found in SEQ ID NO:1 but also having one or more amino acids that differ from the amino acids of SEQ ID NO:1.

Peptide libraries include, for example, tagged chemical libraries comprising peptides and peptidomimetic molecules. Peptide libraries also comprise those generated by phage display technology. Phage display technology includes the expression of peptide molecules on the surface of phage as well as other methodologies by which a protein ligand is or can be associated with the nucleic acid which encodes it. Methods for the production of phage display libraries, including vectors and methods of diversifying the population of peptides which are expressed, are well known in the art (*see, for example,* Smith and Scott, *Methods Enzymol. 217:*228-257 (1993); Scott and Smith, *Science 249:*386-390 (1990); and Huse, WO 91/07141 and WO 91/07149). These or other well known methods can be used to produce a phage display library, from which the displayed peptides can be cleaved and assayed for activity in alleviating neuropathic pain or other neurotrophic or myelinotrophic activity as described herein. If desired, a population of peptides can be assayed for activity, and an active population can be subdivided and the assay repeated in order to isolate an active peptide from the population. Other methods for producing peptides useful in the invention include, for example, rational design and mutagenesis based on the amino acid sequences of active fragments of prosaposin such as SEQ ID NO:1 and SEQ ID NO:2, for example.

As disclosed herein, a prosaposin receptor agonist useful in alleviating neuropathic pain can be identified by its activity in alleviating neuropathic pain in any of a number of well-established animal models of neuropathic pain (Bennett, *supra* (1993)). For example, a prosaposin receptor agonist can be identified using an experimental model of peripheral neuropathy produced by segmental spinal nerve ligation in the rat. The Chung rat model duplicates the symptoms of human patients with causalgia, or burning pain due to injury of a peripheral nerve (Kim and Chung, *supra* (1992)). The surgical procedure of Kim and Chung produces a long-lasting hyperalgesia to noxious heat and mechanical allodynia of the affected foot. As described in EXAMPLE I, rats with spinal nerve ligation according to the procedure developed by Chung and Kim are useful for identifying a prosaposin receptor agonist for use in alleviating neuropathic pain.

A prosaposin receptor agonist useful in alleviating neuropathic pain also can be identified by its activity in alleviating neuropathic pain in a rat model of painful diabetic neuropathy. Hyperalgesia to thermal, mechanical and chemical noxious stimuli also has been reported in diabetic rats with short-term insulin-deficient diabetes induced by selective β cell toxins such as streptozotocin (Calcutt *et al., Pain 68:*293-299 (1996)). Such a rat model is representative of the pain evidenced in diabetic humans, who may exhibit a variety of aberrant sensations including spontaneous pain, pain evoked by light touch and hyperalgesia. Rats treated with streptozotocin or another selective β cell toxin can be treated with a fragment or peptide of interest; subsequently, the response to a noxious stimulus such as 0.5% formalin is measured. A reduced response can be used to identify a prosaposin receptor agonist useful in alleviating neuropathic pain.

A prosaposin receptor agonist useful in alleviating neuropathic pain also can be identified using the neuroma model of Wall *et al.* This well-recognized model of neuropathic pain reproduces the human symptoms seen following amputation or nerve transection in an intact limb (Wall *et al., supra* (1979)). As discussed above, a neuroma forms readily after nerve transection due to the frustrated growth of neurite sprouts.

A model of chronic constriction injury also can be used to identify a prosaposin receptor agonist useful in alleviating neuropathic pain. The chronic constriction injury model of Bennett and Xie,

supra (1988) is a rat model of peripheral neuropathy that produces pain disorders like those seen in man. In the Bennett model, nerve injury is created by loosely tying constrictive ligatures around the rat sciatic nerve, causing degeneration of nerve distal to the constriction. Allodynia and hyperalgesia are produced by the constriction injury in addition to spontaneous pain.

Primate models of neuropathic pain also are useful for identifying a prosaposin receptor agonist (*see, for example,* Lekan *et al., supra* (1992); Palacek *et al., supra* (1992)).

As used herein, the term "peptide," as used in reference to an active fragment of prosaposin, a prosaposin-derived peptide or a peptide useful in the methods of the invention, means a compound containing naturally occurring amino acids, non-naturally occurring amino acids or chemically modified amino acids, provided that the compound retains activity in alleviating neuropathic pain or other neurotrophic or myelinotrophic activity as described herein. A prosaposin receptor agonist also can be a peptide mimetic, which is a non-amino acid chemical structure that mimics the structure of a prosaposin-derived peptide and retains activity. Such a mimetic generally is characterized as exhibiting similar physical characteristics such as size, charge or hydrophobicity in the same spatial arrangement found in the prosaposin-derived peptide counterpart. A specific example of a peptide mimetic is a compound in which the amide bond between one or more of the amino acids is replaced, for example, by a carbon-carbon bond or other bond well known in the art (*see, for example,* Sawyer, *Peptide Based Drug Design*, ACS, Washington (1995)).

As used herein, the term "amino acid" refers to one of the twenty naturally occurring amino acids, including, unless stated otherwise, L-amino acids and D-amino acids. The term amino acid also refers to compounds such as chemically modified amino acids including amino acid analogs, naturally occurring amino acids that are not usually incorporated into proteins such as norleucine, and chemically synthesized compounds having properties known in the art to be characteristic of an amino acid, provided that the compound can be substituted within a peptide such that it retains its biological activity. For example, glutamine can be an amino acid analog of asparagine, provided that it can be substituted within an active fragment of prosaposin that retains its activity in alleviating neuropathic pain or other neurotrophic or myelinotrophic activity as described herein.

Other examples of amino acids and amino acids analogs are listed in Gross and Meienhofer, *The Peptides: Analysis, Synthesis, Biology*, Academic Press, Inc., New York (1983). An amino acid also can be an amino acid mimetic, which is a structure that exhibits substantially the same spatial arrangement of functional groups as an amino acid but does not necessarily have both the α-amino and α-carboxyl groups characteristic of an amino acid.

A prosaposin receptor agonist can be isolated or synthesized using methods well known in the art. Such methods include recombinant DNA methods and chemical synthesis methods for production of a peptide. Recombinant methods of producing a peptide through expression of a nucleic acid sequence encoding the peptide in a suitable host cell are well known in the art and are described, for example, in Sambrook *et al., Molecular Cloning: A Laboratory Manual*, 2nd Ed., Vols. 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989).

A prosaposin receptor agonist useful in the invention also can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield *et al., J. Am. Chem. Soc. 85*:2149 (1964). Standard solution methods well known in the art also can be used to synthesize a peptide useful in the invention (*see, for example,* Bodanszky, *Principles of Peptide Synthesis,* Springer-Verlag, Berlin (1984) and Bodanszky, *Peptide Chemistry,* Springer-Verlag, Berlin (1993)). A newly synthesized peptide can be purified, for example, by high performance liquid chromatography (HPLC), and can be characterized using, for example, mass spectrometry or amino acid sequence analysis.

It is understood that limited modifications can be made to an active fragment of prosaposin without destroying its biological function. Thus, a modification of an active fragment of prosaposin that does not destroy its ability to alleviate neuropathic pain is within the definition of a prosaposin receptor agonist. A modification can include, for example, an addition, deletion, or substitution of amino acid residues; a substitution of a compound that mimics amino acid structure or function; and addition of chemical moieties such as amino or acetyl groups. The activity of a modified peptide in alleviating neuropathic pain can be assayed using an animal model of neuropathic pain, such as those described above or the assay exemplified in EXAMPLE I.

A particularly useful modification of a prosaposin receptor agonist is one that confers, for example, increased stability. For example, incorporation of one or more D-amino acids or substitution or deletion of lysine can increase the stability of an active fragment of prosaposin by protecting against peptide degradation. For example, as disclosed herein, the prosaposin-derived 14-mer SEQ ID NO:2 has an amino acid sequence derived from amino acids 16 to 29 of saposin C but which has been modified by substitution or deletion of each of the three naturally occurring lysines and the addition of a C-terminal tyrosine residue. In particular, the prosaposin-derived 14-mer SEQ ID NO:2 has a D-alanine for lysine substitution at position 2; an alanine for lysine substitution at position 8 and a deletion of lysine at position 11. The D-alanine substitution at position 2 confers increased stability by protecting the peptide from endoprotease degradation, as is well known in the art (*see, for example,* page 247 of Partridge, *Peptide Drug Delivery to the Brain,* Raven Press, New York (1991)). The substitution or deletion of a lysine residue confers increased resistance to trypsin-like proteases, as is well known in the art (Partridge, *supra* (1991)). These substitutions increase stability and, thus, bioavailability of peptide SEQ ID NO:2, but do not affect activity in alleviating neuropathic pain.

A useful modification also can be one that promotes peptide passage across the blood-brain barrier, such as a modification that increases lipophilicity or decreases hydrogen bonding. For example, a tyrosine residue added to the C-terminus of the prosaposin-derived peptide (SEQ ID NO:2) increases hydrophobicity and permeability to the blood-brain barrier (*see, for example,* Banks *et al., Peptides 13:*1289-1294 (1992) and Pardridge, *supra* (1991)). A chimeric peptide-pharmaceutical that has increased biological stability or increased permeability to the blood-brain barrier, for example, also can be useful in the method of the invention.

One skilled in the art can readily assay the ability of a prosaposin receptor agonist to cross the blood-brain barrier *in vivo*, for example, as disclosed in EXAMPLE II. In addition, an active fragment of prosaposin can be tested for its ability to cross the blood-brain barrier using an *in vitro* model of the blood-brain barrier based on a brain microvessel endothelial cell culture system, for example as described in Bowman *et al., Ann. Neurol. 14:*396-402 (1983) or Takahura *et al., Adv. Pharmacol. 22:*137-165 (1992).

As used herein, the term "a neuropathic pain alleviating amount" or "effective amount" means the amount of a prosaposin receptor agonist useful for causing a diminution in neuropathic pain, whether by alleviating neuropathic pain or by inhibiting the onset of neuropathic pain. An effective amount to be administered systemically on a daily basis depends on the body weight of the subject. Preferably, an effective amount to be administered systemically on a daily basis is about 0.1 μg/kg to about 1000 μg/kg. More preferably, an effective amount to be administered systemically on a daily basis is about 10 μg/kg to about 100 μg/kg. An effective amount of a peptide for alleviating or inhibiting the onset of pain can be determined empirically using methods well known to those in the art, including, for example, the assay described in EXAMPLE I or those disclosed above, including assays in primates (Lekan *et al., supra* (1992), and Palacek *et al., supra* (1992)).

A typical minimum amount of the peptides of the invention for neurotrophic or myelinotrophic activity in cell growth medium is at least about 5 ng/ml. This amount or more of a peptide of the invention can be used for *in vitro* use. Typically, concentrations in the range of 0.1 μg/ml to about 10 μg/ml of a peptide of the invention can be used. An effective amount for treatment of a particular tissue can be determined as set forth in EXAMPLES IV and VI.

Neural cells can be treated *in vitro* or *ex vivo* by directly administering a peptide of the invention to the cells. This can be done, for example, by culturing the cells in growth medium suitable for a particular cell type, followed by addition of peptide to the medium. When the neural cells to be treated are *in vivo*, typically in a vertebrate, preferably a mammal, a peptide of the invention can be administered by one of several techniques as described below.

As used herein, the term "subject" means a vertebrate, preferably a mammal and, in particular, a human.

The present invention provides methods of alleviating pain, stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination and inhibiting demyelination and methods of inhibiting sensory or motor neuropathy by administering an effective amount of an active fragment of prosaposin intravenously, intramuscularly, intradermally, subcutaneously, intracranially,

intracerebrospinally, topically, orally, transdermally, transmucosally, or intranasally. A pharmaceutically acceptable carrier of well known type can be administered with a prosaposin receptor agonist. Such carriers include, for example, phosphate buffered saline (PBS).

Preferably, an effective amount of a prosaposin receptor agonist is injected directly into the bloodstream of the subject. For example, intravenous injection of a prosaposin receptor agonist can be used to administer the active fragment to the peripheral or central nervous system, since an iodinated prosaposin-derived 18-mer Tyr-Lys-Glu-Val-Thr-Lys-Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO:20), consisting of amino acids 12 to 29 of prosaposin-derived 22-mer SEQ ID NO:1 with a substitution of tyrosine for valine at amino acid 12 (MW = 2000) crossed the blood-brain barrier and entered the central nervous system as described in EXAMPLE II. The uptake by the brain was approximately 0.03%, which is in the mid-range of values for peptides of that approximate size that will cross the blood-brain barrier (Banks *et al., supra* (1992)).

Oral administration often can be desirable, provided the prosaposin receptor agonist is modified so as to be stable to gastrointestinal degradation and readily absorbable. The substitution, for example, of one or more D-amino acids can confer increased stability to a prosaposin receptor agonist useful in the invention.

Direct intracranial injection or injection into the cerebrospinal fluid also can be used to introduce an effective amount of a prosaposin receptor agonist into the central nervous system of a subject. In addition, a prosaposin receptor agonist can be administered to peripheral neural tissue by direct injection or local topical application or by systemic administration. Various conventional modes of administration also are contemplated, including intravenous, intramuscular, intradermal, subcutaneous, intracranial, epidural, topical, oral, transdermal, transmucosal, and intranasal administration.

A prosaposin receptor agonist also can be administered in a sustained release form. The sustained release of a prosaposin receptor agonist has the advantage of alleviating neuropathic pain over an extended period of time without the need for repeated administrations of the active fragment.

Sustained release can be achieved, for example, with a sustained release material such as a wafer, an immunobead, a micropump or other material that provides for controlled slow release of the prosaposin receptor agonist. Such controlled release materials are well known in the art and available from commercial sources (Alza Corp., Palo Alto CA; Depotech, La Jolla CA; *see, also,* Pardoll, *Ann. Rev. Immunol. 13:*399-415 (1995)). In addition, a bioerodible or biodegradable material that can be formulated with a prosaposin receptor agonist, such as polylactic acid, polygalactic acid, regenerated collagen, multilamellar liposomes or other conventional depot formulations, can be implanted to slowly release the active fragment of prosaposin. The use of infusion pumps, matrix entrapment systems, and transdermal delivery devices also are contemplated in the present invention.

A prosaposin receptor agonist also can be advantageously enclosed in micelles or liposomes. Liposome encapsulation technology is well known. Liposomes can be targeted to a specific tissue, such as neural tissue, through the use of receptors, ligands or antibodies capable of binding the targeted tissue. The preparation of these formulations is well known in the art (*see, for example,* Pardridge, *supra* (1991), and Radin and Metz, *Meth. Enzymol. 98:*613-618 (1983)).

A peptide composition of the invention can be packaged and administered in unit dosage form, such as an injectable composition or local preparation in a dosage amount equivalent to the daily dosage administered to a patient, and if desired can be prepared in a controlled release formulation. Unit dosage form can be, for example, a septum sealed vial containing a daily dose of the active composition of the invention in PBS or in lyophilized form. For treatment of neural diseases, an appropriate daily systemic dosages of a peptide of the invention is based on the body weight of the vertebrate and is in the range of from about 10 to about 100 μg/kg, although dosages from about 0.1 to about 1,000 μg/kg are also contemplated. Thus, for the typical 70 kg human, a systemic dosage can be between about 7 and about 70,000 μg daily and preferably between about 700 and about

7,000 μg daily. A daily dosage of locally administered material will be about an order of magnitude less than the systemic dosage. Oral administration is also contemplated.

The invention also provides a method of alleviating neuropathic pain in a subject by transplanting into the subject a cell genetically modified to express and secrete a prosaposin receptor agonist. Transplantation can provide a continuous source of a prosaposin receptor agonist and, thus, sustained alleviation of neuropathic pain. For a subject suffering from prolonged or chronic neuropathic pain, such a method has the advantage of obviating or reducing the need for repeated administration of an active fragment of prosaposin.

Using methods well known in the art, a cell readily can be transfected with an expression vector containing a nucleic acid encoding an active fragment of prosaposin (Chang, *Somatic Gene Therapy,* CRC Press, Boca Raton (1995)). Following transplantation into the brain, for example, the transfected cell expresses and secretes an active fragment of prosaposin and, thus, alleviates neuropathic pain. Such a method can be useful to alleviate neuropathic pain as described for the transplantation of cells that secrete substances with analgesic properties (*see, for example,* Czech and Sagen, *Prog. Neurobiol. 46:*507-529 (1995)).

The cell can be any cell that can survive when transplanted and that can be modified to express and secrete an active fragment of prosaposin. In practice, the cell should be immunologically compatible with the subject. For example, a particularly useful cell is a cell isolated from the subject to be treated, since such a cell is immunologically compatible with the subject.

A cell derived from a source other than the subject to be treated also can be useful if protected from immune rejection using, for example, microencapsulation or immunosuppression. Useful microencapsulation membrane materials include alginate-poly-L-lysine alginate and agarose (*see, for example,* Goosen, *Fundamentals of Animal Cell Encapsulation and Immobilization,* CRC Press, Boca Raton (1993); Tai and Sun, *FASEB J. 7:*1061 (1993); Liu *et al., Hum. Gene Ther. 4:*291 (1993); and Taniguchi *et al., Transplant. Proc. 24:* 2977 (1992)). For example, pain reduction has

been achieved using polymer encapsulated cells transplanted into the rat spinal subarachnoid space (Wang *et al., Soc. Neurosci. Abstr. 17:*235 (1991)).

For treatment of a human subject, the cell can be a human cell, although a non-human mammalian cell also can be useful. In particular, a human fibroblast, muscle cell, glial cell, neuronal precursor cell or neuron can be transfected with an expression vector to express and secrete an active fragment of prosaposin such as SEQ ID NO:1. A primary fibroblast can be obtained, for example, from a skin biopsy of the subject to be treated and maintained under standard tissue culture conditions. A primary muscle cell also can be useful for transplantation. Considerations for neural transplantation are described, for example, in Chang, *supra* (1995).

A cell derived from the central nervous system can be particularly useful for transplantation to the central nervous system since the survival of such a cell is enhanced within its natural environment. A neuronal precursor cell is particularly useful in the method of the invention since a neuronal precursor cell can be grown in culture, transfected with an expression vector and introduced into an individual, where it is integrated. The isolation of neuronal precursor cells, which are capable of proliferating and differentiating into neurons and glial cells, is described in Renfranz *et al., Cell 66:*713-729 (1991).

Methods of transfecting cells *ex vivo* are well known in the art (Kriegler, *Gene Transfer and Expression: A Laboratory Manual,* W.H. Freeman & Co., New York (1990)). For the transfection of a cell that continues to divide such as a fibroblast, muscle cell, glial cell or neuronal precursor cell, a retroviral vector is preferred. For the transfection of an expression vector into a postmitotic cell such as a neuron, a replication-defective herpes simplex virus type 1 (HSV-1) vector is useful (During *et al., Soc. Neurosci. Abstr. 17:*140 (1991); Sable *et al., Soc. Neurosci. Abstr. 17:*570 (1991)).

A nucleic acid encoding an active fragment of prosaposin can be expressed under the control of one of a variety of promoters well known in the art, including a constitutive promoter or inducible promoter. *See, for example,* Chang, *supra* (1995). A particularly useful constitutive promoter for

high level expression is the Moloney murine leukemia virus long-terminal repeat (MLV-LTR), the cytomegalovirus immediate-early (CMV-IE) or the simian virus 40 early region (SV40).

A nucleic acid sequence encoding an active fragment of prosaposin is disclosed herein. For example, a nucleic acid sequence encoding SEQ ID NO:1 is 5'-TGTGAATTCCTGGTGAAGGAGGTGACCAAGCTGATTGACAACAACAAGACTGAGAAAGAAATACTC-3' (SEQ ID NO:21) (Dewji *et al., Proc. Natl. Acad. Sci. USA 84:*8652-8656 (1987)). In order to direct secretion of peptide SEQ ID NO:1, for example, a nucleic acid encoding a signal sequence, such as the signal sequence of β-lactamase, can be operably linked to SEQ ID NO:21 as described in Simon *et al., J. Cell Biol. 104:*1165 (1987).

The invention further provides a method of inhibiting the onset of neuropathic pain in a subject by administering an effective amount of a prosaposin receptor agonist to the subject. The method of preventing neuropathic pain is useful when applied prior to a painful event, for example, prior to chemotherapy or surgery that is known to result in neuropathic pain.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I
Alleviation of Neuropathic Pain in Chung Model Rats

This EXAMPLE describes the effects of bolus intrathecal injection of an active fragment of prosaposin in the Chung experimental model of peripheral neuropathic pain.

Each of the three peptides were obtained in pure form by chemical synthesis, dissolved in sterile PBS and buffered to a neutral pH.

The surgical procedure previously described by Kim and Chung, *supra* (1992) was performed on male Sprague-Dawley rats weighing 120 to 150 grams to induce an allodynic state. Briefly, the rats were anesthetized with halothane; subsequently, the left L-5 and L-6 spinal nerves were isolated adjacent to the vertebral column and ligated with 6.0 silk suture distal to the dorsal root ganglion.

After a ten to fourteen day post operative recovery period, a spinal catheter was introduced. Five days following the second surgery, intrathecal drug administration was accomplished using a gear driven micro-injection syringe connected to a spinal catheter inserted through the foramen magnum. Prior to testing, the rats were placed in clear plastic wire meshed cages and allowed to acclimate.

To assess the 50% mechanical threshold for paw withdrawal, a von Frey hair was applied to the hind foot avoiding the foot pad. Each of the von Frey hairs, which are calibrated to bend at increasing log forces, were pressed perpendicularly to the foot with sufficient force to cause slight bending for a duration of approximately six to eight seconds. A positive response was noted if the foot was sharply withdrawn. Six data points were collected for each point with the maximum and minimum stimulus noted for each time point. The resulting pattern of the responses was tabulated, and the 50% response threshold was computed. The graph gives the response to the indicated dosage of peptide given as a single intrathecal bolus injection. The X-axis indicates the time after the injection at which point the hypersensitivity to pressure on the foot pad was measured.

All surgically lesioned rats showed tactile allodynia prior to injection with an active fragment of prosaposin. As shown at time zero in FIGURE 1, the measured threshold was less than 3.0 to 4.0 g in the absence of peptide. Intrathecal injection of 0.7 or 0.07 μg of the prosaposin-derived 22-mer peptide (SEQ ID NO:1) suppressed allodynia in a dose-dependent fashion. The reduction of allodynia is manifest by the increase in the force threshold as the rats withstand an increasing force before withdrawing the affected foot.

A significant effect was observed by 15 minutes after the injection. The maximum effect was seen 120 minutes post-injection. Rats injected with the highest dose of the prosaposin-derived 22-mer peptide (SEQ ID NO:1) continued to demonstrate significantly reduced allodynia at the latest time point assayed (180 minutes). Rats that were injected with 0.007 μg prosaposin-derived 22-mer peptide (SEQ ID NO:1) showed no significant reduction in allodynia. No significant side effects such as sedation were observed at any concentration.

The ability of the prosaposin-derived 14-mer peptide (SEQ ID NO:2; *see* TABLE 1) to relieve allodynia in Chung model rats also was examined. As shown in FIGURE 2, the active fragment of prosaposin (SEQ ID NO:2) was effective in reducing allodynia. The peak effect of the prosaposin-derived 14-mer peptide (SEQ ID NO:2) was observed 15 to 30 minutes following the injection and returned to the pre-injection value by 60 minutes (FIGURE 2). No side effects were observed at either concentration of prosaposin-derived 14-mer peptide (SEQ ID NO:2) tested.

A mutant 22-mer peptide (SEQ ID NO:8) that differs from the prosaposin-derived 22-mer peptide (SEQ ID NO:1) by containing an aspartic acid residue instead of an asparagine (*see* TABLE 4) also was tested for activity in relieving allodynia in Chung model rats. No change in the allodynic response of the Chung rats was observed following injection of 17.5 μg mutant 22-mer peptide (SEQ ID NO:8).

Normal rats, which do not experience pain as a result of surgical lesion introduced according to the Chung model, also were injected with an active fragment of prosaposin (SEQ ID NO:1) and tested for their response to a heat stimulus according to the procedure developed by Bennett and Xie, *supra* (1988). Briefly, the period of time before the rat withdraws the affected foot from a source of heat is defined as the hot plate latency and is a measure of tolerance to pain caused by a heat stimulus.

An intrathecal catheter was placed into normal male Sprague Dawley rats. Five days after this surgery, rats were injected intrathecally with an active fragment of prosaposin (SEQ ID NO:1). Rats were examined on the hot plate (52.5°C); hot plate response latencies were measured prior to injection and at various time points up to 180 minutes after the injection. No significant elevation of the hot plate response latency was observed. Thus, the prosaposin-derived peptide SEQ ID NO:1 does not effect the perception of pain in normal animals.

EXAMPLE II
In vivo Uptake of Prosaposin-derived Peptides By the Central Nervous System

The results described in this EXAMPLE indicate that prosaposin-derived peptides cross the blood-brain barrier.

An 18-mer peptide (SEQ ID NO:20) consisting of amino acids 12-29 of saposin C with a tyrosine substituted for valine at position 12 was chemically synthesized on an Applied Biosystems Model 430 peptide synthesizer. The peptide was then radioiodinated by the lactoperoxidase method; 20 x 10^6 cpm radiolabeled peptide were injected into the auricles of rats. The animals were sacrificed after one hour and 24 hours, and the hearts were perfused with isotonic saline in order to remove the blood from the brain.

In order to determine the percentage of peptide uptake, the brain was then counted in a gamma counter. In addition, the brain was homogenized and fractionated into a capillary rich fraction (pellet) and a parenchymal brain fraction (supernatant) after dextran centrifugation (Triguero *et al.*, *J. Neurochem.*, *54:*1882-1888 (1990)). This method allows for the discrimination between radiolabeled peptide within blood vessels and that within the brain. After 24 hours, 0.017% of the injected peptide (SEQ ID NO:20) was detected in whole brain; 75% of the label was in the parenchymal fraction and 25% was in the capillary fraction. At 1 hour, 0.03% of the injected dose was present in whole brain.

The prosaposin-derived peptide SEQ ID NO:2 also was assayed for ability to cross the blood-brain barrier as follows. A female Sprague-Dawley rat was anesthesized with methoxyflurane, and approximately 20 μg peptide SEQ ID NO:2 (3.2 x 10^8 cpm) was injected into the tail vein. After 40 minutes, the rat was sacrificed by ether anesthesia and perfused with about 250 ml PBS through the heart. The total amount of peptide in brain, liver and blood was calculated as a percentage of the injected material as shown in TABLE 6. In order to determine the localization in brain, the capillary depletion method of Triguero, *J. Neurochem.* *54:*1882 (1990) was used to separate brain tissue into a parenchyma fraction and a brain capillary fraction. The fractionation results showed

that 87% of the SEQ ID NO:2 peptide present in brain was localized to brain parenchyma while 13% was found in brain capillary.

TABLE 6			
TISSUE	**WEIGHT**	**TOTAL CPM IN TISSUE**	**PERCENTAGE OF INITIAL CPM**
Brain	1.3 gm	161,000	0.050
Liver	8.8 gm	5.2×10^6	1.625
Blood	about 22μl	1.01×10^8	31.6

In a similar experiment in which rats were sacrificed after three hours treatment with SEQ ID NO:2, 0.06% of the peptide was evident in brain, of which 85% was in the parenchyma. These results demonstrate that at least some of the prosaposin-derived peptide SEQ ID NO:2 crossed the blood brain barrier and was concentrated in the brain parenchyma rather than the vascular endothelium (blood vessels). The percentage of peptide which crossed the blood brain barrier is in the mid-range of peptides which cross the barrier as set forth in Banks, *supra* (1992).

In order to determine the percentage of intact material in the brain, liver and blood, radiolabeled material (SEQ ID NO:2) isolated from the tissues was analyzed by high pressure liquid chromatography. To normalize for degradation during processing of tissue homogenates, peptide SEQ ID NO:2 was added to tissue homogenates. The extent of degradation observed with the added peptide material was used to normalize for degradation during tissue processing. After normalization, the results were as follows: SEQ ID NO:2 was about 60% intact in brain; about 80% intact in liver and about 40% intact in blood. In a second experiment, peptide SEQ ID NO:2 was about 68% intact in brain. These results indicate that the peptide SEQ ID NO:2 crosses the blood brain barrier and is largely intact in brain.

EXAMPLE III
Alleviation of Neuropathic Pain in Diabetic Rats

This EXAMPLE describes the effects of intraperitoneal administration of a peptide having the sequence of SEQ ID NO:2 in a rat model of diabetic neuropathy.

Rats were made diabetic by a single intraperitoneal injection of streptozotocin (STZ) (50 mg/kg body weight, freshly dissolved in 0.9% sterile saline) to ablate pancreatic β cells and induce insulin deficiency as described in Calcutt *et al., Pain 68:*293-299 (1996). Two days later, diabetes was confirmed in streptozotocin-injected rats by measuring blood glucose levels. Streptozotocin-injected animals with a blood glucose concentration below 15 mmol/l were excluded from subsequent studies, according to the commonly accepted definition of non-fasting hyperglycemia in studies of diabetes in rats.

Both diabetic and control rats were studied at 8 weeks by analyzing the behavioral response to the noxious chemical formalin as an indicator of allodynia (Calcutt *et al. supra* (1996)). Briefly, rats received a subcutaneous injection of freshly-prepared formalin (50 μl of 0.5% solution in sterile saline) into the dorsal surface of the right hind paw. This concentration of formalin induces sub-maximal behavioral responses in control rats and allows detection of hyperalgesia in diabetic rats during phases Q and 2 (Calcutt *et al., Eur. J. Pharmacol. 285:*189-197 (1995). Animals were transferred to an observation chamber constructed to allow continuous visualization of the paws. The number of flinches during one minute periods were counted at 5 minute intervals for the next 60 minutes by an observer who was unaware of the treatment group of each animal. Phase 1 was defined as the initial measurement of flinching (1-2 and 5-6 minutes post injection); the Q (quiescent) phase as the measurements made at 10-11, 15-16 and 20-21 minutes; and Phase 2 as all subsequent measurements post injection, as previously defined for studies of diabetic rats (*see, for example,* Malmberg *et al., Neurosci. Lett. 161:*45-48 (1993)). Comparisons of activity during each phase were made by summing the flinches at measurement points within the phase. Diabetic rats gave an abnormal flinch response, as has been reported previously.

Peptide SEQ ID NO:2 was obtained in pure form by chemical synthesis, dissolved in sterile PBS and buffered to a neutral pH. Diabetic rats were divided in two groups of four animals each, which were administered saline or peptide SEQ ID NO:2, respectively. Two hours before treatment with 0.5% formalin, the diabetic rats were treated with saline or 200 μg/kg peptide SEQ ID NO:2 using intraperitoneal administration. As shown in FIGURE 3, administration of SEQ ID NO:2 completely prevented the abnormal flinch response in Phase 1 and ameliorated the response in Phase 2 by 70%. Thus, parenteral administration of peptide SEQ ID NO:2 alleviated the pain from formalin injection in a rat model of painful diabetic neuropathy.

EXAMPLE IV
Stimulation of Neurite Outgrowth *in vitro*

This EXAMPLE describes the use of a peptide having the sequence of SEQ ID NO:2 in stimulating neurite outgrowth *in vitro*.

NS20Y neuroblastoma cells are grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS). Cells are removed with trypsin and plated in 30 mm petri dishes onto glass coverslips. After 20 to 24 hours, the medium is replaced with 2 ml DMEM containing 0.5% fetal calf serum with 0, 0.5, 1, 2, 4 or 8 ng/ml of a peptide having sequence SEQ ID NO:2 or a scrambled control peptide. Cells are cultured for an additional 24 hours, washed with PBS and fixed with Bouin's solution (saturated aqueous picric acid/formalin/acetic acid 15:5:1) for 30 minutes. After fixative is removed with PBS, neurite outgrowth is scored under a phase contrast microscope. Cells exhibiting one or more clearly defined neurites equal to or longer than one cell diameter are scored as positive for neurite outgrowth. At least 200 cells are scored in different positions of each dish to determine the percentage of neurite bearing cells with each peptide assayed in duplicate.

The peptide shown in SEQ ID NO:2 significantly increases neurite outgrowth in NS20Y cells as compared to a scrambled control peptide having the same amino acids in a different order. Increased neurite outgrowth is evident using as little as 0.5 ng/ml peptide.

EXAMPLE V
Inhibition of Neural Cell Death *in vitro*

This EXAMPLE describes the use of a peptide having the sequence of SEQ ID NO:2 in inhibiting neural cell death *in vitro*.

NS20Y cells are plated as described in EXAMPLE IV and grown on glass coverslips in 0.5% fetal bovine serum for 2 days in the presence or absence of 8 ng/ml of a peptide having the sequence shown as SEQ ID NO:2 or a scrambled control peptide. Media is removed and 0.2% trypan blue in PBS is added to each well. Dead cells stain blue with the trypan blue dye and are scored as a percentage of the total on an inverted microscope, counting 400 cells in four areas of each well. The average error of duplicates is ±5%. The peptide shown as SEQ ID NO:2 substantially reduces the number of trypan blue-positive (dead) cells. This indicates that a peptide having the sequence SEQ ID NO:2 can inhibit programmed cell death.

EXAMPLE VI
Ex vivo Myelination Assay

This EXAMPLE describes the use of a peptide having the sequence of SEQ ID NO:2 in stimulating neurite outgrowth *ex vivo* and in promoting myelination.

Newborn mouse cerebellar explants are prepared according to Satomi, *Zool. Sci. 9:*127-137 (1992). Neurite outgrowth and myelination are observed over 22 days in culture, during the period when the newborn mouse cerebellum normally undergoes neuronal differentiation and myelination begins. On the second day after preparation of the explants, the peptide having the sequence of SEQ ID NO:2 is added to three explants at a concentration of 10 μg/ml and a scrambled control peptide is added to three explants at a concentration of 10 μg/ml. Neurite outgrowth and myelination in three control and three treated explants is assessed under a bright field microscope with a video camera. On the eighth day, cultures containing the peptides are thinner and more spread out than control cultures. On day 15, cultures treated with peptide SEQ ID NO:2 contain many cells with long projections at the periphery of the explant. Such projections are absent or less prominent in control cultures. Cultures treated with peptide SEQ ID NO:2 contain significantly more myelinated axons

in the subcortical white matter at 22 days compared to control explants. Thus, the peptide of the invention induces myelination in differentiating cerebellum *ex vivo*.

EXAMPLE VII
Inhibition of Demyelination

Reduction of Schwann cell death is correlated with inhibition of demyelination. Schwann cells contain an extensive myelin sheath. The addition of the peptide shown in SEQ ID NO:2 to Schwann cells in culture reduces Schwann cell death in a dose-dependent manner not seen with a control scrambled peptide. Thus, a peptide of the invention having the sequence of SEQ ID NO:2 can inhibit demyelination.

EXAMPLE VIII
Treatment of Traumatic Ischemic CNS Lesions

Humans with traumatic lesions to the spinal cord receive an intracerebrospinal injection or direct injection of about 100 μg/ml of the peptide shown in SEQ ID NO:2 in a sterile saline solution or in depot form to enable slow, continuous release of the peptide at the lesion site. Improvement is assessed by gain of motor nerve function such as increased limb movement. Treatments are repeated until no further improvement occurs.

EXAMPLE IX
Treatment of Demyelination Disorders

Patients diagnosed with early stage MS are given a peptide having the sequence shown in SEQ ID NO:2 by direct intravenous injection into the cerebrospinal fluid using the same dose range as in EXAMPLE VIII. Dosages are repeated daily or weekly and improvement in muscle strength, musculoskeletal coordination and myelination (as determined by magnetic resonance imaging (MRI)) is observed.

EXAMPLE X
Treatment of Sensory Neuropathy

Mice were administered taxol in order to induce sensory neuropathy. The taxol-treated mice were administered 100 μg/kg, 200 μg/kg or 1 mg/kg of the prosaposin-derived peptide SEQ ID NO:2.

The loss of thermal sensation was measured using a Hargreaves sensory testing apparatus as an indicator of sensory neuropathy. Each of the three doses of peptide SEQ ID NO:2 administered were effective in inhibiting loss of thermal sensation in taxol-treated mice. The prosaposin-derived 22-mer peptide SEQ ID NO:1 also was similarly assayed and found to be effective in inhibiting less of thermal sensation in the taxol-treated mice. These results show that prosaposin-derived peptides such as SEQ ID NO:1 and SEQ ID NO:2 can be used to effectively inhibit sensory neuropathy.

EXAMPLE XI
Incorporation of ^{32}P into NS20Y Proteins After Treatment with Prosaposin or its Active Fragments

NS20Y cells were incubated in phosphate-free Hanks' balanced salt solution containing 2.5 μg/ml actinomycin D and 80-100 μCi/ml carrier-free [^{32}P]-orthophosphate (New England Nuclear) and effector proteins (0.5-1.0 μg/ml) and incubated for 10-15 minutes at room temperature. Cells were solubilized in SDS-PAGE sample buffer, analyzed by SDS-PAGE and autoradiographed.

Prosaposin and saposin C were found to stimulate phosphorylation of proteins of 148, 100, 80, 68, 50, 38 and 34 kDa to a greater extent than controls or cells treated with similar concentrations of saposins A, B or D. This 148 kDa protein may be phospholipase C-γ, a protein known to be involved in phospholipid metabolism and which is phosphorylated on tyrosine residues in response to a number of growth factors. Densitometric analysis indicated a 3-5 fold stimulation of phosphorylation after 10 minutes. Treatment of gels with alkali revealed that the prominent phosphorylated proteins were alkali-resistant, indicating that they contain phosphotyrosine and/or phosphothreonine (located next to proline) residues. These results indicate that prosaposin and its active fragments bind to a cell surface receptor and activate a kinase cascade, similar to other neurotrophins and growth factors. Since prosaposin-ganglioside GM_1 or saposin C-ganglioside GM_3 complexes inhibit neuritogenesis, while prosaposin or saposin C alone promote this process, this indicates that gangliosides may abolish neurotogenic activity by masking a receptor binding site on the neurotrophin. In addition, since prosaposin and its active fragments induce tyrosine phosphorylation of cytoplasmic proteins in responsive cells, most likely by activation of a tyrosine

kinase similar to cytokines and growth factors, this provides further evidence that a cell surface receptor is involved.

A 20 kDa protein has been identified as the putative receptor for prosaposin as described in the following EXAMPLE:

EXAMPLE XII
Isolation of a Putative Prosaposin Receptor

A putative prosaposin receptor protein was isolated from whole rat brain, rat cerebellum and mouse neuroblastoma cells using the plasma membrane P-100 fraction. Briefly, cells or tissues were solubilized and centrifuged at 14,000 rpm to remove debris. The supernatant was centrifuged at 40,000 rpm for 1 hour at 4°C. The pellet, enriched in plasma membrane, was solubilized in RIPA buffer (10 mM MOPS, pH 7.5, 0.3 M sucrose, 5 mM EDTA, 1% Trasylol, 10 μM leupeptin and 10 μM antipain). This P-100 fraction was applied to an affinity column containing the bound, active 22-mer fragment of saposin C. The column was washed with 0.05 M NaCl to elute loosely-bound proteins followed by 0.25 M NaCl which eluted the putative 54-60 kDa prosaposin receptor. In addition, it was determined that the 54-60 kDa protein could be eluted using a 100-fold excess of unbound peptide thus demonstrating specific elution. The 54-60 kDa protein was approximately 90% pure as judged by SDS-PAGE. The protein was purified to homogeneity using HPLC and eluted at 50% acetonitrile in an acetonitrile/water gradient on a Vydac C4 column. After treatment with the cross-linking reagent disuccinimidyl suberate (DSS; Pierce, Rockford, IL), the 54-60 kDa protein bound irreversibly to ^{125}I labeled saposin C as evidenced by the 72 kDa molecular weight of the complex (60 kDa + 12 kDa).

EXAMPLE XIII
Alleviation of Neuropathic Pain in Selzer Model Rats

Prosaptide TX 14(A) (SEQ ID NO:2) was tested for relief of hyperalgesia in the Selzer rat model. After induction of hyperalgesia by ligation of the sciatic nerve in one hind limb, the animals were injected with Prosaptide TX 14(A). Relief was measured using the hot plate withdrawal technique comparing ligated vs. unligated hind foot withdrawal. Within 3 hours of injection of Prosaptide TX

14(A), intravenous at a dose of 200 mg/kg, nearly complete relief of hyperalgesia was observed. The relief lasted for up to 48 hours post injection.

EXAMPLE XIV
The Effects of Prosaptide TX 14(A) on the Relief of Neuropathy in a Diabetic Rat Model

While conventional medical treatment of diabetes mellitus markedly prolongs life-span, serious medical complications affect a large proportion of the more than 10 million people believed to have diabetes in the USA. Peripheral neuropathy is the most common complication, affecting one third of newly diagnosed cases. The frequency of neuropathy increases with duration of disease to affect over half of all diabetics. Nerve dysfunction usually progresses to become a distal symmetrical polyneuropathy with morphologic evidence of paranodal widening, segmental demyelination and remyelination, axonal atrophy and ultimate fiber loss. This pathology is accompanies by loss of sensory function that, when coupled with impaired healing processes and vascular disease, can lead to gangrene and limb amputation.

Hyperglycemia may be induced in animals by a variety of means and diabetic rodents have been widely studied to gain insights into mechanisms underlying diabetic complications. Acute, insulin-deficient, experimental diabetes may be introduced by selective chemical ablation of the beta cells of the pancreas using streptozatocm (STZ) to produce analogous to severe type 1 (insulin-dependent) diabetes.

STZ-diabetic rats exhibit electrophysiologic disorders that are similar to those found in newly-diagnosed diabetic patients. Symptoms include, reduced nerve conduction, relacitios, and resistance to ischemic conduction block. Other functional disorders present in the peripheral nerves of STZ-diabetic rats include exaggerated pain responses to a painful stimulus with concurrent thermal hypoalgesia (slowing of response times to painful thermal stimuli, reduced levels of neuropeptide neurotransmitters, impaired regeneration after injury and reduced nerve blood).

Since prosaposin and the prosaptides have been shown to cause peripheral and central nerve regeneration and prevent demyelination and induce remyelination, this preliminary EXAMPLE was conducted using prosaptide TX 14(A) (SEQ ID NO:2) using STZ-diabetic rats.

Control and STZ-diabetic rats were treated using 1000 μg prosaptide TX 14(A) per kg body weights i.p., three times per week for 8 weeks. Treatment did not prevent hyperglycemia or loss of body weight in diabetic rats (control = 280 gm; control plus prosaptide TX 14(A) = 280 gm; diabetic = 195 gm; and diabetic plus prosaptide TX 14(A) = 204 gm). However, prosaptide TX 14(A) prevented hypoalgesia in the STZ-diabetic rats (FIGURE 4).

This is an important finding as loss of thermal sensation and thermal pain sensation is an early indicator of neuropathy in diabetic patients.

EXAMPLE XV
Prosaposin in Peripheral Nerve: Effects of Diabetes and Efficacy of a Peptide Fragment of Prosaposin in Treating Diabetic Nerve Dysfunction

A more comprehensive EXAMPLE was then undertaken to include more parameters and to establish a dose response of prosaptide TX 14(A) (SEQ ID NO:2). In this EXAMPLE, control and STZ-diabetic rats were treated with prosaptide TX 14(A) at doses of 20 μg/kg, 200 μg/kg, and 1000 μg/kg body weight, i.p., three times per week for 8 weeks. The rats were weighed at the beginning, midpoint and the end of the experiment and motor nerve conduction (MNCV) and sensor nerve conduction velocity (SNCV) measurements were taken at the three time points. In this EXAMPLE, all three groups treated with prosaptide TX 14(A) showed significant decreases in weight loss in a dose dependent manner when compared to diabetic animals (FIGURE 5). Thermal response latency was restored to control non-diabetic levels in animals treated with 200 and 1000 μg prosaptide TX 14(A), while the 20 μg treatment group showed an intermediate response (FIGURE 5). Diabetic animals treated with prosaptide TX 14(A) at doses of 200 and 1000 μg/kg body weight showed enhanced motor nerve conduction velocities compared to the untreated diabetic group and diabetic animals receiving 20 μg/kg body weight (FIGURE 6). In addition, all prosaptide TX 14(A)-treated

rats showed a slower loss of sensory nerve conduction velocity when compared to diabetic unheated animals (FIGURE 6).

In detail, the results of this EXAMPLE were as follows: Saposin C and its precursor prosaposin exhibit neurogenic properties and protect neurons from ischemic injury. Immunostaining, using a monoclonal antibody that recognizes both prosaposin and saposin C, was demonstrated in Schwann cells of peripheral nerve. Further, after 8 weeks of untreated diabetes, prosaposin mRNA levels increased 2-fold in the peripheral nerves of rats, suggesting either dysfunctional processing or a local response to developing neuropathy. Therefore, the effect of a 14 amino acid neuroactive peptide fragment of saposin C (prosaptide; SEQ ID NO:2) on peripheral nerve function in control and diabetic rats was investigated. MNCV (63.7 ± 1.0 m/s;: mean term) and SNCV (63.4 ± 1.3) were significantly ($p<0.05$ or less by ANOVA and Student-Newman-Keuls test) reduced after 8 weeks of streptozotocin diabetes (52.8 ± 1.1 and 49.8 ± 1.8 respectively). These deficits were attenuated by thrice weekly treatment (1 mg/kg i.p. for 8 weeks) with prosaptide (58.1 ± 1.2 and 54.9 ± 1.3 respectively). Thermal hypoalgesia in diabetic rats (control = 85 ± 3.4 sec: diabetic = 11.0 ± 0.8) was also prevented by prosaptide treatment (8.8 ± 0.6) while there was no effect on hyperglycemia, accumulation of polyol pathway metabolites, nerve myo-inositol depletion or reduced nerve laser Doppler flux. There was no detectable effect of prosaptide on any measured parameter in control rats. In a subsequent time course study, thermal hypoalgesia and the decline in nerve conduction velocities during 8 weeks of diabetes were attenuated by prosaptide in a dose-dependent manner using thrice weekly treatment with either 20,. 200 or 1000 μg i.p. These data demonstrate the novel presence in Schwann cells of a neuroactive protein (prosaposin) whose mRNA is increased during diabetes. Moreover, a peptide fragment of this protein (prosaptide; SEQ ID NO:2) is capable of preventing slowed nerve conductance and thermal hypoalgesia in diabetic rats without modulating exaggerated polyol pathway flux or reduced nerve vascular perfusion.

This EXAMPLE demonstrates that prosaptide TX 14(A) (SEQ ID NO:2) can prevent the symptoms of diabetic neuropathy and slow or prevent motor and sensory nerve degeneration as measured by nerve conduction velocities. In addition, the optimal dose of prosaptide can now be established as greater than 20 μg/kg and less than 100 μg/kg body weight.

EXAMPLE XVI

Prosaptide Halts Progressive Slowing of Sensory Conduction And Reverses Hyperalgesia in Diabetic Rats

The results of this EXAMPLE demonstrate that the prevention of formalin-induced pain (allodynia) in diabetic rats treated with prosaptide at 200 mg/kg. The formalin testing was done after 4 weeks, of three time weekly injections, and testing was done after the last dose (last bar, FIGURE 7). In a second study after 8 weeks of diabetes, prosaptide TX 14(A) (SEQ ID NO:2) was administered 30 min before testing (200 mg/kg i.p.) and reversed the hyperalgesia to a significant ($p>0.05$) extent (third bar, FIGURE 7). These results demonstrated that prosaptide TX 14(A), given parenterally, reverses hyperalgesia in the diabetic rat, when given as a single dose after 2 months of neuropathy and latter animal testing was performed 24-48 hours after the last dose, and reversal was again highly significant ($p>0.05$).

In detail, the results of this EXAMPLE show that prosaptide (SEQ ID NO:2), a 14 amino acid neuroactive peptide fragment of saposin C, attenuates the decline in nerve conduction velocities (NCV) of diabetic rats in a dose-dependent manner when given from the onset of diabetes. This EXAMPLE was designed to determine whether prosaptide could also reverse conduction deficits and hyperalgesia in the formalin test pain model once these disorders are established in diabetic rats. SNCV was measured before onset of diabetes and 4-8 weeks later in groups of control, untreated diabetic and prosaptide-treated (200 μg/kg i.p. thrice weekly for the last 4 weeks) diabetic rats. Flinch responses to injection of formalin (50 μl 0.5% or 5.0% solution) were followed for 60 min in control, 8 week untreated diabetic, 8 week diabetic treated with prosaptide (200 μg/kg i.p.) 30 min pre-test and 8 week diabetic treated with prosaptide (200 μg/kg i.p.) thrice weekly for the last 4 weeks (the last treatment being 48-72 hr pre-test). Untreated diabetic rats showed a progressive decline in SNCV so that values were significantly ($P>0.05$ by ANOVA with Dunnett's test) lower than controls after 4 weeks and decreased further between weeks 4 and 8. Treatment with prosaptide beginning after 4 weeks of diabetes prevents any further decline in SNCV between weeks 4 and 8 of diabetes. These animals also showed a significant ($P>0.05$ vs. untreated diabetic rats by ANOVA) reduction in hyperalgesia during the formalin test compared to untreated diabetics. A single bolus treatment with prosaptide given 30 min before testing in otherwise untreated 8 week

diabetic rats was also effective in abolishing hyperalgesia during the formalin test whereas prosaptide in single bolus doses of up to 1 mg/kg i.p. 30 min pre-test was without effect on responses to formalin in control rats.

This EXAMPLE shows that the progression of an established SNCV disorder in diabetic rats can be halted by prosaptide. The anti-hyperalgesic properties of prosaptide are specific to diabetes rats rather than being a general effect on the formalin test *per se,* and prosaptide is effective either as a single dose 30 min pre-test or by chronic treatment with the final dose being at least 48 hr pre-test.

Although the invention has been described with reference to the EXAMPLES above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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